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Short communication

Identification of euglenoid algae that produce ichthyotoxin(s)

P V Zimba[1], M Rowan[2] and R Triemer[3]

1 USDA/ARS/MSA/CGRU, Stoneville, MS, USA
2 Vernon James Research Center, North Carolina State University, Plymouth, NC, USA
3 Department of Plant Biology, Michigan State University, East Lansing, MI, USA

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Reported incidences of harmful algal blooms (HABs) have increased during the past 20 years, suggesting either increased occurrence or more rigorous monitoring efforts (Landsberg 2002). Several toxic compounds are produced, including neurotoxins, hepatotoxins, dermonecrotics and/or gastroenterics. Direct HAB effects include animal mortalities from toxin ingestion and/or oxygen depletion following algal senescence. Indirect impacts from HABs include alterations of normal trophic transfer resulting from food web alterations, fish mortalities from secondary stressors (disease/indirect oxygen effects) and lost revenue from tourist and recreational industry cancellations (Anderson, Kaoru & White 2000). Diatoms, dinoflagellates, pelagiophytes and prymnesiophytes are the only divisions of microalgae known to produce toxins (Wehr & Sheath 2003); cyanobacteria additionally are known to produce at least three classes of toxins (Chorus 2001).

Most toxin-producing taxa occur in marine/estuarine systems; but herein we describe the production of an unknown toxin by freshwater *Euglena* species. Unexplained fish mortalities were reported in July–August 2002 at a commercial aquaculture facility in North Carolina. Fish mortalities were reported from three 1.2 ha striped bass, *Morone saxatilis* (Walbaum), ponds; two of these ponds had total mortality. Over 21 000 striped bass died and revenue lost from this single event exceeded $100 000. No clear pathology was evident in dead fish, with the exception of reddening of the gill tissue. No water quality factors (nitrates, ammonia, pH, temperature) were abnormal and dissolved oxygen was above 4 mg L^{-1} during fish mortalities. Water samples from the second mortality event examined microscopically contained >99% *Euglena* by numeric counts. When exposed to the pond water, laboratory-reared channel catfish, *Ictalurus punctatus* (Rafinesque), were killed within 6.5 min.

Pond water was serially fractionated to separate dissolved compounds, bacteria, and algal fractions using 0.22, 2.0 and 10 μm nitrocellulose filters (Costar, Cambridge, MA, USA). Both dissolved and algal fractions were toxic to fish. The dissolved fraction maintained toxicity following treatment with a proteinase, suggesting the compound was non-protein. The toxin was stable when heated to 30 °C for 10 min and retained activity when frozen at −80 °C for 60 days.

Euglena cells were isolated from the water samples and brought into unialgal culture in AF-6 media (http://www.nies.go.jp/biology/mcc/medium.htm#AF-6). Light microscopic analyses verified the species identity as *E. sanguinea*, a cosmopolitan species found in many shallow, relatively calm, eutrophic freshwater systems (Fig. 1a).

Correspondence *Dr P V Zimba, USDA/ARS/MSA/CGRU, PO Box 38, Stoneville, MS 38776, USA (e-mail: pzimba@msa-stoneville.ars.usda.gov)*

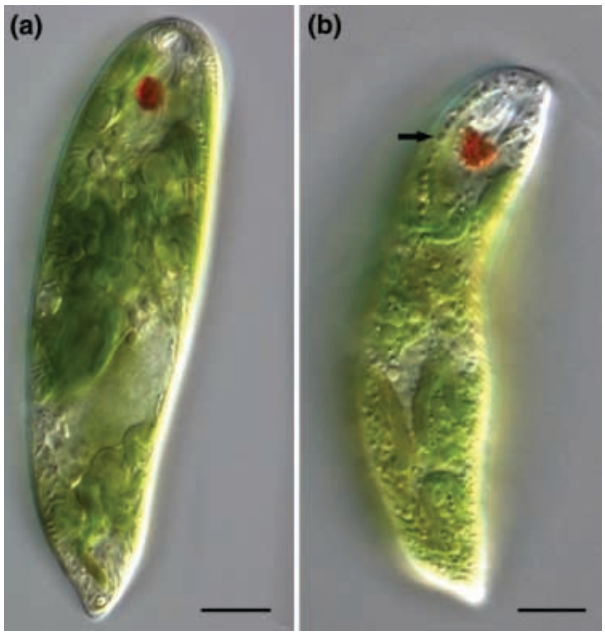

Figure 1 Toxin producing photosynthetic euglenoids. (a) *Euglena sanguinea* isolated from a freshwater pond in North Carolina. (b) University of Texas Culture Collection strain LB2345, now identified as *E. granulata*. One of the many rows of subsurface mucocysts is seen at the arrow (bars = 10 μm).

Fish tested with cultured *E. sanguinea* cells and with filtrate from the cultures exhibited disorientation during exposure, with accelerated respiration and an inability to maintain equilibria. Gill tissue was reddened, however no distinct haemorrhaging was evident. No other distinct histopathology was seen. We suggest that the toxin functions as a neurotoxin based upon the behavioural changes.

Juvenile catfish exposed to cultures of the algal isolates died within 2 h of exposure (Table 1). Toxic activity was highest within the <3000 Da size range as determined using Centricon cutoff filters (Millipore Corporation, Billerica, MA, USA). Mortality occurred following intraperitoneal injection (0.25 mL filtrate) as well as whole body immersion. Chemical fractionation of algal cells and filtrate to serially separate hydrophobic to hydrophilic constituents confirmed toxicity in the water-soluble fraction (toxicity assessed by sheepshead minnow assay). Identification of the toxin structure is ongoing and will be presented elsewhere.

The potential for strain-specific differences in toxin production was assessed by comparing toxicity of the NC strain with the University of Texas Culture Collection strain LB2345. Both were grown in AF-6 media as previously described. Fish exposed to the algal culture displayed distress and mortality as previously observed. Mortality occurred within 4 h of exposure (Table 1).

Microscopic examination of both algal strains revealed several dissimilar features (Fig. 1b). Chloroplasts in the field isolate had typical ribbon-like chloroplasts, whereas the LB2345 culture con-

Table 1 Fish killing potential of *Euglena* spp. in laboratory and field samples

Date	Location	Fish killed	Sample type	Algal density (cells mL^{-1})
July–August	Field	Striped bass, *Morone saxatilis*	Wild (*E. sanguinea*)	Unknown
Early August	Laboratory	Tilapia, *Oreochromis niloticus*	Wild (*E. sanguinea*)	Unknown
August	Laboratory	Catfish, *Ictalurus punctatus*	Wild (*E. sanguinea*)	3500[a]
November	Laboratory	Catfish, *I. punctatus*	Isolate (*E. sanguinea*)	982
December	Laboratory	Catfish, *I. punctatus*	Culture (UTEX LB2345)	1220
January	Laboratory	Sheepshead, *Cyprinodon variegatus*	Culture (UTEX LB2345)	1345[b]

[a] Sample also contained over 1500 resting cysts.
[b] Sample also contained over 1100 resting cysts.

tained chloroplasts that were more disc-shaped. Furthermore, spirally arranged rows of mucocysts under the cell surface indicated that the organisms in this culture belonged to *E. granulata* and not *E. sanguinea*, demonstrating that at least two species of *Euglena* produce toxins.

This is the first report of fish kills by any freshwater algal taxa. The evidence for toxin production by *E. sanguinea* and *E. granulata* is unambiguous. *Euglena sanguinea* is a very common, cosmopolitan alga that has been reported from all continents except Antarctica. *Euglena granulata* is also widely reported in temperate and tropical freshwater systems. Both grow best in eutrophic environments. Increased nutrification of wetlands, use of lagoonal style wastewater treatment systems, and runoff from urbanization (hardening) will increase suitable habitat for growth of these algae with consequent risks of economic loss.

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